Influence of Nitrate, Phosphate and Herbicide Stresses on Nitrogenase Activity and Growth of Cyanobacteria Isolated from Paddy Fields*

Gulten OKMEN (Kurucuoglu)[1] Gonul DONMEZ[2] Sedat DONMEZ[3]
[1] Mugla University, Faculty of Science and Arts, Department of Biology, Mugla, TURKEY
[2]Ankara University, Faculty of Science, Department of Biology, Ankara, TURKEY
[3]Ankara University, Faculty of Engineering, Food Engineering, Ankara, TURKEY

Corresponding author:
e-mail : gultenokmen@gmail.com
* This study is a unit of Ph.D.Thesis
Received : 20 June 2006
Accepted : 11 August 2006

Abstract

Samples were collected from paddy fields in Corum-TURKIYE. Nitrogen-free BG-11 medium was used for isolation of nitrogen fixing cyanobacteria. Acetylene reduction technique was used to determine the effects of different chemical agents on the nitrogenase activities of the cyanobacteria, which were identified at the genus level. *Nodularia* showed the highest nitrogenase activity (0.006 ethylene µl/ mg.h) at 10mM nitrate concentration. At 25mM phosphate concentration, *Nodularia* showed the highest nitrogenase activity (0.006 ethylene µl/ mg.h). The highest tolerances for the herbicides were present in *Nodularia* (0.06 ethylene µl/ mg.h) for bensulphuron (50µg /ml) and *Nostoc* 6 ethylene µl/ mg.h (for molinate 100µg / ml).

Key words: Cyanobacteria, nitrogenase activity, isolation, environmental factors

INTRODUCTION

The utilization of nitrogen gas (N_2) as a source of nitrogen is called nitrogen fixation and it is a property of only certain prokaryotes [1, 2]. Soil algae, particularly nitrogen fixing cyanobacteria, are important photosynthetic microorganisms because they contribute to soil fertility by fixing the atmospheric nitrogen.

In the fixation processs, N_2 is reduced to ammonium and the ammonium is converted to the organic form. The reduction process is catalyzed by the nitrogenase which consists of two separate proteins called dinitrogenase and dinitrogenase reductase [2, 3]. Nitrogenase activity is controlled by a complex regulon called the nif regulon [4, 5].

Biological processes contribute 65 % of the nitrogen used in agriculture [6]. Biological nitrogen fixation contributions to rice culture up to 75kgN ha^{-1} per culture cycle [7]. Free living microorganisms on temperate soil and waters are thought to fix as much as 45-100kg N ha^{-1} yr^{-1} only cyanobacteria fix as much as 28 kg N ha^{-1} yr^{-1}[8]. More over, biofertilizers have been more important because algalization may be effect plant size, nitrogen content and the number of tillers, ears, spikelets and filled grains per panicle.

Certain photosynthetic bacteria fix N_2, but only under anaerobic conditions. The nitrogen fixation has been affected by environmental factors. Nitrate, phosphate and herbicide stresses are an important environmental factors affecting algal growth and nitrogenase activity.

Singh [9] suggested that cultures of *Nostoc* sp. rapidly and significantly lost their ability to reduce acetylene when incubated with 2mM NH_4Cl and 5mM glutamine in light. Prosperi et al. [10] determined that the input of nitrogen fertilizers to field reduces nitrogen fixation since the presence of combined nitrogen inhibits nitrogenase activity , also they researched the repressive effect of ammonium on nitrogenase activity at neutral pH but not at alkaline pH, and it is so-called "fast switch-off". Singh et al. [11] found that nitrogenase and heterocyst were repressed by NH_3 at *Anabaena cycadeae*. Juan et al. [12] reported that transfer of N_2-fixer filamentous cyanobacteria from media containing a source of combined nitrogen to a medium lacking combined nitrogen provokes the differentiation of heterocyst, specialized cells able to perform dinitrogen fixation [13, 14]. Meeks et al. [15] informed that both of species of *Anabaena* sp were maximum inhibited of acetylene reduction activity and heterocyst formation between 25 and 100µM (69% and 36%), and they did not increase at higher nitrate concentrations. Moisander and Pearl [16] explained that dissolved inorganic N is a factor because it inhibits nitrogenase. Sroga [17] also indicated that nitrogenase activity of *Microcoleus* sp. was inhibited by NO_3, NH_3, urea under the light and dark phase. Anneliese et al. [18] reported that the effection of inhibitory of NH_3 haven't been under the anaerobic conditions. Jose et al. [19] determined that nitrogenase structural genes and some other genes related to dinitrogen fixation repression by ammonium and different degrees of inhibition have been reported for different strains at nitrate. Bottomley et al . [20] revealed that both of NH_4NO_3 and KNO_3 completely repressed heterocyst development and nitrogenase activity at *Anabaena* sp. Valiente et al. [21] found that there was a negative correlation between ammonium and nitrogenase activity and the activation of nitrogenase was sharply inhibited. Turpin et al. [22] reported that 1mM ammonium at all pH was repressed the nitrogenase activity on *Anabaena flos-aquae* and at higher pH, the proportion of unprotonated ammonia increases and diffusion across the call membrane can occur.

Adhya et al. [23] said that phosphorus is one of macronutrient essentials for plant growth, and addition of P to rice fields promotes root growth and rhizosphere activity and heterotrophic nitrogen fixation. Wilson and Alexander [24] established that phosphate equivalent to 30kgP/ha stimulated nitrogen fixation by about 60%, and the growth of nitrogen fixing algae was also limited in flooded paddy fields. According to Turid [25], phosphorus fertilization stimulated the nitrogenase activity, but to some other researchers; it was repressed [26, 27]. Lehtimaki et al. [28] observed that growth of *Nodularia* sp. incubation in different phosphorus concentrations was barely detetable during first 21 days. Huber [29] found that the rate of more or less than 0.9 µM the phosphate concentration is the best condition for akinet growth at *Nodularia* sp. Leganes et al. [30] established that grain yielding on paddy field stimulated at 100kgP/ha treatments .

Jianyi et al. [31] searched on the effect of 40 herbicides on *Chlorella vulgaris* and they were determined that the photosynthetic period of *Chlorella vulgaris* was effected by molinate and the acetolactatsintase of *Chlorella vulgaris* was

effected by bensulfuron – methyl. Yan et al. [32] researched that the effects of molinate at *Anabaena sphaerica* on 300-3000 lux (5, 25, 50µg/ml) and some specific proteins were prevented functionally by toxic effect. Mansour et al. [33] and Caux et al. [34] demonstrated that toxic effect of molinate is more effective at low light intensity (300lux) than high light intensity (3000lux) and it was related to organic carbon s which was more assimilated in this condition.

Rice cultivation in India started in assured irrigation areas during the rainy summer season before 25 to 30 years ago [35]. Herbicides used in rice are categorized into preplant, preemergence and postemergence [36 - 38]. The role of environmental factors on nitrogenase activity is not known yet. Because of this it needs to work on it. This paper summarizes effects of this nitrate, phosphate and herbicide stresses on growths and nitrogenase activities of nitrogen-fixing *Anabaena*, *Nostoc* and *Nodularia* sp.

MATERIALS AND METHODS

Materials

The filamentous, heterocystous cyanobacteria were used in this study in which *Anabaena*, *Nodularia* and *Nostoc* sp. which were isolated from soil with water samples obtained from rice fields in Corum, Türkiye. *Nostoc* and *Nodularia* strains were obtained from previous studies of Prof. Dr. Gonul Donmez.

Isolation and purification were performed by dilution and plating of soil and water samples. Stock cultures were grown in the N-free BG-11 medium as previously described [10]. Temperature was maintained at 20°C and cultures were grown under a cool white light (600lux). Cells in the logarithmic phase of growth were collected from stock cultures and used as inocula for experiments.

Experiments were conducted in batch cultures by using 10 ml of inoculated medium in 25ml. Erlenmyer flasks enclosed with cotton plugs. Culture media were adjusted accordingly pH (7, 8, 9) with 1N NaOH and 1N HCl. Illumination was supplied with 600lux cool white light [39 - 41].

Methods

Determination of nitrogenase activity

Nitrogenase activity was measured by acetylene reduction technique using in 10 ml aliquots of cell suspensions placed in stoppered 35 ml serum bottles [42]. Cultures were grown under the different environmental conditions were enclosed by plastic plugs and parafin, then 1ml of acetylene gase was injected into the serum bottles.

Cultures were incubated for 12h under the experiment conditions. After the incubation periods, samples (1ml) were taken from serum bottles with gas-tight syringes, injected into the gas chromatograph, and ethylene concentrations were determined using a Shimadzu GC-14B.

Determination of dry weight

The pellets of centrifuged cultures were washed with distilled water three times, then dried to constant weight at 70°C for 12h [10, 43]. Dry weight were measured.

Influence of nitrate, phosphate and herbicides on nitrogenase activity and growth

The influence of different concentrations of KNO_3 (0.5mM-50mM), K_2HPO_4 (10µM-1M), bensulphuron-methyl (50-500 µg/ml) and molinate (5-50µg/ml) on the nitrogenase activity were also tested on *Anabaena*, *Nostoc* and *Nodularia*.

The experimental cultures were grown in 25ml flasks containing 10ml N-free BG-11 medium under the same conditions as described below. According to Rippka [41], the axenic cultures were grown in a liquid sterilized medium at 20 ± 2 °C under fluorescent light (600lux) for 35days. At the end of 35 days, nitrogenase activity of cultures was determined using the acetylene reduction technique. For dry weight was made as determination described by Cappucino et al. [43]. All experiments were performed in triplicate and parallel conditions.

RESULTS

When *Anabaena*, *Nostoc* and *Nodularia* sp was cultured in the presence of various nitrate, phosphate and herbicide concentrations, distinct effects were seen on nitrogenase activities and growths.

Effects of nitrate on nitrogenase activity and growth

The growths and nitrogenase activities of *Anabaena*, *Nostoc* and *Nodularia* sp treated with different concentrations of nitrate under 600 lux light intensity are listed in Table1. It can be seen that the nitrate markedly inhibited the growths and nitrogenase activities of all cultures. The inhibitory effect increased with the increase in nitrate concentration. Under 100mM nitrate concentration, the nitrogenase activities of all cultures were completely reduced. The highest nitrogenase activity of *Nostoc* sp at different concentration were registered with 1 mM nitrate (0.12µl ethylene / mg.h). The lowest nitrogenase activity of *Nodularia* sp at different concentration were found with 10mM nitrate (0.006µl ethylene / mg.h). The growths of *Anabaena* and *Nostoc* sp. completely repressed at 10mM, but the growth of *Nodularia* sp supressed at 100mM nitrate concentration.

Table 1. Effects of nitrate on nitrogenase activity and growth of cyanobacteria **

Treatment	*Anabaena* sp.		*Nostoc* sp.		*Nodularia* sp.	
	Dry weight (mg/l)	Ethylene amount (µl / mg.h)	Dry weight (mg/l)	Ethylene amount (µl / mg.h)	Dry weight (mg/l)	Ethylene amount (µl / mg.h)
Control	520±52	0,37±0,05	30±1,5	11±2,13	280±28	0,74±0,24
10 µM	470±15	0,003±0	22±1,4	0,17±0	290±10	0,36±0,011
100 µM	396±5,7	0,003±0	17±1,4	0,16±0,007	115±7,07	0,016±0,0007
1 mM	225±7,07	0,003±0	15±1,4	0,12±0,021	90±5,7	0,008±0,0006
10 mM	0	0	0	0	50±0	0,006±0,0007
100 mM	0	0	0	0 0 0		
1M	0	0	0	0 0 0		

** Nitrate effects on the growth ($p < 0.01$).

Effects of phosphate on nitrogenase activity and growth

The effect of phosphate on nitrogenase activities and growths of all cultures shown (Table2). *Anabaena* and *Nostoc* sp. were shown to tolerance to 10mM phosphate concentration and *Nodularia* sp. was shown to tolerance to 25mM. Nitrogenase activity of *Anabaena* sp. was stimulated at 500µM phosphate concentration but increasing

concentrations repressed the nitrogenase activity. In *Nostoc* and *Nodularia* sp., the activities repressed with increasing phosphate concentrations during the initial period. The growths of *Anabaena* and *Nostoc* sp. completely repressed at 25mM and higher phosphate concentrations, and *Nodularia* sp. completely suppressed at 50mM phosphate concentration (Table2).

Table 2. Effects of phosphate on nitrogenase activity and growth of cyanobacteria **

Treatment	*Anabaena* sp.		*Nostoc* sp.		*Nodularia* sp.	
	Dry weight (mg/l)	Ethylene amount (µl / mg.h)	Dry weight (mg/l)	Ethylene amount (µl / mg.h)	Dry weight (mg/l)	Ethylene amount (µl / mg.h)
Control 540±80		0,26±0,06	29±3,6	14,6±1,15	280±28	0,72±0,22
500 µM	500±20	0,45±0,08	25±4,5	0,03±0,02	160±0	0,002±0
5 mM	355±7,07	0,40±0	26±4,6	0,02±0	153±5,7	0,002±0
10 mM	296±23	0,30±0	22±3,6	0,02±0	125±7,07	0,002±0
25 mM	0	0	0	0	113±20,8	0,002±0
50 mM	0	0	0	0	0	0

****** Phosphate effects on the growth ($p < 0.01$).

Effects of bensulfuron-methyl on nitrogenase activity and growth

Table 3 summarise the effects of bensulfuron- methyl concentrations on all of the cultures. The maximum tolerance (0.06µl ethylene / mg.h) was seen at *Nodularia* sp. in 50µg/ml bensulfuron- methyl concentration. In *Anabaena* and *Nostoc* sp., the tolerances were found in 40 µg/ml bensulfuron- methyl concentration.

Although a low bensulfuron-methyl concentration (5µg/ml) somewhat stimulated nitrogenase activity, at higher concentrations nitrogenase activity was severely inhibited at *Anabaena* sp. In *Nodularia* sp., the nitrogenase activity inhibition with bensulfuron-methyl at 5µg/ml was severely. For *Nostoc* sp., the highest nitrogenase activity was seen at 30 µg /ml bensulfuron-methyl. The negative impact of high bensulfuron-methyl on the biomass of all cultures was also shown.

Table 3. Effects of bensulfuron-methyl on nitrogenase activity and growth of cyanobacteria **

Treatment (µg/ml)	*Anabaena* sp.		*Nostoc* sp.		*Nodularia* sp.	
	Dry weight (mg/l)	Ethylene amount (µl / mg.h)	Dry weight (mg/l)	Ethylene amount (µl / mg.h)	Dry weight (mg/l)	Ethylene amount (µl / mg.h)
Control 420±20		0,24±0,04	45±5,6	6,25±0,9	210±10	1±0,2
5 480±30		0,8±0,03	50±1,5	5,8±1,0	145±7,07	0,09±0
10 450±10		0,25±0,03	41±3,5	4,6±0,2	143±5,7	0,08±0,011
20 415±35		0,26±0,014	30±0,7	4,5±0	130±14	0,07±0,02
30 250±30		0,25±0,02	19±2,6	4,5±0	115±7,07	0,07±0,007
40 0		0	0	0	105±7,07	0,07±0
50 0		0	0	0	90±10	0,06±0,006

**Bensulfuron-methyl effects on the growth ($p < 0.01$).

Effects of molinate on nitrogenase activity and growth

The results in Table 4 show that the nitrogenase activities and growths decreased at all molinate levels. The minimum activity was de termined at *Anabaena* sp. (0.12µl ethylene / mg.h) whereas, the highest activity was shown at *Nostoc* sp. (6µl ethylene / mg. h). The maximum tolerance were seen at all of cultures in 100µg/ml molinate concentration.

Molinate experiments have shown that the initial nitrogenase activity of *Nodularia* sp. at low concentration of molinate (50µl / ml) does not change. The nitrogenase activities of all cultures completely repressed at 200 µg/ml molinate concentration.

Table 4. Effects of molinate on nitrogenase activity and growth of cyanobacteria **

Treatment (µg/ml)	*Anabaena* sp.		*Nostoc* sp.		*Nodularia* sp.	
	Dry weight (mg/l)	Ethylene amount (µl / mg.h)	Dry weight (mg/l)	Ethylene amount (µl / mg.h)	Dry weight (mg/l)	Ethylene amount (µl / mg.h)
Control	420±20,0	0,24±0,04	43±5,6	8,15±1,7	365±7,07	0,27±0,014
50	406±20,8	0,14±0,04	31±1,7	7±0,45	180±14,00	0,26±0,014
100	320±30,0	0,12±0,035	27±2,0	6±0,25	150±0,00	0,20±0,035
200	0	0	0	0	0	0
300	0	0	0	0	0	0
500	0	0	0	0	0	0

** Molinate effects on the growth ($p < 0.01$).

DISCUSSION

As stated in the introduction, soil algae are grown in different environmental factors. Variation in growth conditions influenced the growths and nitrogenase activities of all genera. Nitrate is an important one that affects the algal growth. Generally, the addition of nitrate inhibited both the algal growth and nitrogenase activity. All nitrogenase activities were sharply repressed and the algal growth was partly inhibited by nitrate (Table 1).

This confirms the report by Huber [44] for the *Nodularia*, and it is similar to the reports Bottemley et al.[20], who found that the nitrogenase activity of *Nodularia* suppressed by the addition of nitrate.

According to the literature, the maximal inhibition of acetylene reduction and heterocyst formation in *A. cylindrica* occurred between and 25 and 100 µM and did not increase at higher nitrate concentrations [15]. Its results are similar to those of our studies. These results can be explained in this way: the nitrogenase activity inactivated by nitrate, which resembles the so-called " switch-off", observed in phototrophic bacteria.

The comparison of nitrogenase activities of algal cells under the different phosphate concentration, the nitrogenase activity of *Anabaena* sp. stimulated at 500µM phosphate concentration, whereas the nitrogenase activities of the other two species inhibited (Table 2).

In addition, the algal growths of all the cultures were partly suppressed. These results may be described like the following: phosphate is necessary for the algal growth but it is not necessary for the nitrogenase activity [23]. According to the research [25], phosphorus fertilization stimulated the nitrogenase activity and the highest activity was obtained with about 300 µM (200 µE/m^2.s) at *Anabaena* sp., also the nitrogenase activity of *Nostoc* sp. stimulated at 12mM phosphate concentration, however more phosphate concentrations repressed the nitrogenase activity, the result of which is similar to this study. In *Nostoc* and *Nodularia* sp., the nitrogenase activities inhibited at the beginning (Table2). These results seem to suggest that phosphorus stimulated nitrogenase activity in P- starved cells but not in P- suffient cells [44].

It is anonymously reported that [45], bensulfuron-methyl and molinate are mostly used for eliminating weeds in paddy fields in Corum- Osmancık in Turkiye. For this reason, two herbicides were chosen for this study. In herbicide treatments, bensulfuron- methyl stimulated nitrogenase activity of *Anabaena* sp. at 5µg/ml but not in higher concentrations. Whereas the nitrogenase activities and growths of other two species were inhibited during the initial concentration (5µg/ml) (Table3), it was demonstrated that *Anabaena* sp. was capable of growing both photoautotrophically and photoheterotrophically like bacteria to a great extent [32, 46].

In molinate treatments, all genera demonstrated to tolerance to 100 µg/ml level of molinate concentration. In addition, the nitrogenase activities and growths of all genera completely repressed with an increase in molinate concentration. Yan et al.[32] reported that *A. sphaerica* kept growth rate at 100 µg/ml molinate concentration. This result is similar to our studies.

Most reports demonstrated that the inhibitory effect of herbicide became greater with an increase in herbicide concentration and suggested that the reduction in the growth rate of algae may be due to a decrease in algal photosynthesis caused by the inhibition of synthesis of chlorophyll, the most important pigment in algal cells for collecting solar energy for photosynthesis [47, 48].

The data obtained in this study provide information about the inhibitory effect of the different environmental factors on growths and nitrogenase activities of all genera, under which the cyanobacteria exhibits different sensitivity to the factors. These findings suggest a ban on the use of molinate and bensulfuron- methyl in paddy fields, owing to its inhibitory effect. Moreover, these results showed that nitrate and phosphate fertilizers could be applied under lower concentrations to rice fields.

Several differences in the growth and nitrogenase activity rates of *Nodularia*, *Nostoc* and *Anabaena* sp. were observed, which may explain the different vertical, horizontal and temporal distribution of the three genera in paddy fields. In this study, we have shown a clear physiologic distinction between *Nostoc* sp. and the other strains. Generally *Nostoc* sp. had the best optimal performance of nitrogenase activity in all environmental conditions, so it is thought that it is a suitable genus for biofertilizer. A better understanding of the mechanisms require further study about the nitrate, phosphate and herbicide stresses.

REFERENCES

[1]. Manahan SE. 1997. The Nitrogen Cycle. In: Environmental Science and Technology, Lewis Publishers, N.York, 466-468.

[2]. Madigan MT, Martinko JM and Parker J. 1997. Brock Biology of Microorganisms. Prentice-Hall Int. Ltd. 8th edition. In: Chapter 4, pp. 13,14, and 15, London.
[3]. Vignais PM. *et al.* 1985. Biochemistry of nitrogenase. Advances in Microbial Physiology, 26: 190-234.
[4]. Postgate J. 1989. Trends and perspectives in nitrogen fixation research. Advance in Microbial Physiology, 30: 1-21.
[5]. Herrero A, Muro-Pastor AM and Flores E. 2001. Nitrogen control in cyanobacteria. Journal of Bacteriology, 183 (2): 411-425.
[6]. Albrecht SL. 1998. Eukaryotic algae and cyanobacteria. In: Principles and Applications of Soil Microbiology, Prentice-Hall, Inc., America, 94-103.
[7]. Irisarri P, Gonnet S and Monza J. 2001. Cyanobacteria in Uruguay and rice fields: diversity, nitrogen fixing ability and tolerance to herbicides and combined nitrogen. Journal of Biotechnology, 91 (3): 95-103.
[8]. Metting B. 1990. Micro algae applications in agriculture. Developments in Industrial Microbiology, 31: 265-270.
[9]. Singh S. 1991. Involvement of ammonium assimilation in ammonium inhibition of nitrogenase activity in cyanobacterium, *Nostoc* Anth. Indian Journal of Experimental Biology, 29 (6) : 496-497.
[10]. Prosperi C, Luna C and Valiente EF. 1993. Influence of pH light intensity and oxygen on the short-term effect of ammonium on nitrogenase activity of cyanobacteria from rice fields. Environmental and Experimental Botany, 33 (4): 545-552.
[11]. Singh HN, Rai UN, Rao VV and Bagchi SN. 1983. Evidence for ammonia as an inhibitor of heterocyst and nitrogenase formation in the cyanobacterium *Anabaena cycadeae*. Biochemical and Biophysical Research Communications, 111(1): 180-187.
[12]. Juan LR, Francisco M and Miguel GG. 1985. Regulation of nitrogenase levels in *Anabaena* sp. and other filamentous cyanobacteria Archives of Microbiology, 141:105-111.
[13]. Haselkorn R. 1978. Hetero cysts. Annual Review of Plant Physiology and Plant Molecular Biology, 29: 319-344.
[14]. Stewart WDP. 1980. Some aspects of structure and function in N_2 fixing cyanobacteria. Annual Review Microbiology, 34:497-536.
[15]. Meeks JC, Wycoff KL, Chapman JS and Enderlin CS. 1983. Regulation of expression of nitrate and dinitrogen assimilation by *Anabaena* species. Applied and Environmental Microbiology, 45 (4): 1351-1359.
[16]. Moisander PH and Paerl HW. 2000. Growth, primary productivity and nitrogen fixation potential of *Nodularia* spp. in water from a subtropical estuary in the United States. Journal of Phycology, 36: 645-658.
[17]. Sroga GE. 1997. Regulation of nitrogen fixation by different nitrogen sources in the filamentous non-heterocystous cyanobacterium *Microcoleus* sp. FEMS Microbiology Letters, 153: 11-15.
[18]. Anneliese E, Reich S, and Böger P. 1990. Modification of dinitrogenase reductase in the cyanobacterium *Anabaena variabilis* due to C starvation and ammonia. Journal of Bacteriology, 172 –2: 748-755.
[19]. Jose MN, Herrero A and Flores E. 1991. Control of nitrogenase mRNA levels by products of nitrate assimilation in the cyanobacterium *Anabaena* sp. strain PCC7120. Plant Physiology, 97: 825-828.
[20]. Bottomley PJ, Grillo JF, Balen CV and Tabita FR. 1979. Synthesis of nitrogenase and heterocysts by *Anabaena*, sp. CA in the presence of high levels of ammonia. Journal of Bacteriology, 140 –3: 938-943.
[21]. Valiente EF, Quesada A, Prosperi C, Nieva M, Leganes F and Ucha A. 1997. Short and long term effects of ammonium on photodependent nitrogen fixation in wetland rice fields of Spain. Biology and Fertility of Soils, 24: 353-357.
[22]. Turpin DH, Edie SA and Canvin DT. 1984. In vivo nitrogenase regulation by ammonium and methylamine and the effect of MSX on ammonium transport in *Anabaena flos-aquae*. Plant Physiology, 74: 701-704.
[23]. Adhya TK, Pattnaik P, Satpathy SN, Kumaraswamy S, and Sethunathan N. 1998. Influence of phosphorus application on methane emission and production in flooded paddy soils Soil Biology & Biochemistry, 30-2: 177-181.
[24]. Wilson JT and Alexander M. 1979. Effect of soil nutrient status and pH on nitrogen-fixing algae in flooded soils. Soil Science Society of America Journal, 43: 936-939.
[25]. Turid L. 1999. Environmental factors influencing the nitrogen fixation activity of free- living terrestrial cyanobacteria from a hight arctic area. Canadian Journal of Microbiology, 45-(7): 573-581.
[26]. Basilier K, Granhall U and Stenström TA. 1978. Nitrogen fixation in wet minetrophic moss communities of a subarctic mire. Oikos, 31: 236-246.
[27]. Chapin DM, Bliss LC and Bledsoe LJ. 1991. Environmental regulation of nitrogen fixation in a high arctic lowland ecosystem. Canadian Journal of Botany, 69: 2744-2755.
[28]. Lehtimaki J, Moisander P, Sivonen K and Kononen K. 1997. Growth, nitrogen fixation and nodularin production by two Baltic Sea cyanobacteria. Applied and Environmental Microbiology, 63 (5): 1647-1656.
[29]. Huber AL. 1985. Factors affecting the germination of akinetes of *Nodularia spumigena*. Applied and Environmental Microbiology, 49 (1):73
[30]. Leganes F, Carreres R, Tome RG, Nieva M, Quesada A, Sendra J and Valiente EF. 2001. Effect of phosphate fertilisation, straw incorporation, insecticide application and inoculation with cyanobacteria on rice productivity. Invest. Agr. Prod. Prot.Veg., 16 (2): 273-282.
[31]. Jianyi M, Ligen X, Shufeng W, Rongquan Z, Shuihu J, Songqi H and Youjun H. 2002. Toxicity of 40 Herbicides to the Green Alga *Chlorella vulgaris*. Exotoxicology and Environmental Safety, 51: 128-132.
[32]. Yan GA, Yan X and Wu W. 1997. Effects of the herbicide molinate on mixotrophic growth, photosynthetic pigments and protein content of *Anabaena sphaerica* under different light conditions. Ecotoxicology and Environmental Safety, 38 (2): 144-149.
[33]. Mansour FA, Soliman, ARI., Shaaban-Desouki, SA. and Hussein, MH. 1994. Effect of herbicides on cyanobacteria. I. Changes in carbohydrate content,Pmase and GOT activities in *Nostoc* sp. and *Anabaena* sp. Phykos, 33:153-162.
[34]. Caux PY., Menard L. and Kent RA. 1996. Comparative study of the effects of MCPA, butylate, atrazine and cyanozine on *Selenastrum* of sp. Environmental Pollution, 92: 219-225.

[35]. Narwal SS. 2000. Weed Management in Rice. Critical Review in Plant Sciences, 19(3): 249-266.
[36]. Gianessi LP, Silvers CS, Sankula S and Carpenter JE. 2002. Herbicide Toler ant Rice. Plant Biotechn ology: Current and P otential Imp act for Improving Pest Management in U.S. Agricu lture an analy sis of 40 Case Studies.(www. ncfap. Org), 1-14.
[37]. Pratley JE, Bro ster JC, Flower GE and Flower RF. 2001. Herbicide resistance in the rice growing r egions of Southern Australia. A report for the Rural Industries Research and D evelopment Cor poration, No: 0 1/ 40: 1-11.
[38]. Singh LJ and Tiwari DN. 1988. Effects of selected rice field herb icides on photos ynhesis, respiration and nitrogen assimilating enzy me s ystems of pa ddy soil diazotrophic cynanobacteria. Pesticide B iochemistry and Physiology, 31: 120-128.
[39]. Castenholz RW. 1988. Culturing method s for cyanobacteria. Methods in Enzymology, 167: 68-113.
[40]. Fogg GE, Stewart WDP, Fay P and Walsby AE. 1973. Culture, nutrition and growth . The Blue Green Algae, Academic Press, London, NewYork. 129-142.
[41]. Rippka R. 19 88a. Isolation and purification of cyanobacteria. Methods in Enzymology, 167: 3-27.
[42]. Burlage RS, Atlas R, S tahl D, Geesey G, and Sayler G., 1998. Tech niques in Micr obial Ecolog y Oxford University Press, America, 8-14.
[43]. Cappuccino JG and Sherman N. 2001. Microbiology A Laboratory M anual, Sixth Edition , Benjam in Cummings, 119, S. Francisco.
[44]. Huber AL 1986. Nitrogen fixation b y *Nodularia spumigena* Mertens. 2: Laboratory studies. Hydrobiologia, 133: 193-202.
[45]. Anonymus. 2002. Weed management in the cultured plants growing regions of Corum. Head- Office of Agriculture, page 1, Ankara, Turkiye.
[46]. Jin CY, Song LR and Li SH. 1 996. The mixotr ophic growth of *Anabaena* sp. Acta Hy drobiol. Sin. 2: 134-137.
[47]. Pandey AK. 19 85. Effects of p ropanil on grow th and cell constitu ents of *Nostoc calciola.* Pe sticide Biochemistry and Physiology, 23:157-162.
[48]. Abou- Waly H , Abou- Setta MM, Nigg HN and Mallory LL. 1 991. Growth response of freshwater algae, *Anabaena flos- aquae* and *Selenastrum capricornutum*, to atrazine and hexazinone herbicides. Bulletin of Environmental Contaminatio n and Toxicology, 46: 223 - 229.